

This listing of claims will replace all prior versions, and listing, of claims in the application:

Listing of Claims:

1.-2. (Canceled)

3. (Currently amended) A nucleic acid ligation assay, comprising:

contacting a sample suspected of containing one or more target nucleic acid sequences with one or more subsets of free probes and one or more subsets of spectrally-addressable bound probes;

allowing the one or more subsets of free probes and the one or more subsets of spectrally-addressable bound probes to hybridize to the one or more target nucleic acid sequences, if present in the sample;

ligating the hybridized free probes with the hybridized spectrally-addressable bound ~~probe~~probes, wherein a free probe hybridized to a target nucleic acid sequence is ligated with a spectrally-addressable bound probe hybridized to the same target nucleic acid sequence, to provide spectrally-addressable ligated products; and

~~one or both of detecting the presence of the spectrally-spectrally-addressable ligated products, or analyzing the target nucleic acid sequence sequences of the spectrally-addressable ligated products, or performing said detecting and said analyzing;~~ and wherein:

~~the sample is suspected of containing the one or more target nucleic acid sequences comprise one or more first and one or more second target nucleic acid sequences, wherein the one or more first target nucleic acid sequences have at least a first portion and a second portion, and wherein the one or more second target nucleic acid sequences have at least a first portion and a second portion, and wherein the first portion of the one or more first target nucleic acid sequences is distinguishable from the first portion of the one or more second target nucleic acid sequences, but and wherein the second portion of the one or more first target nucleic acid sequences is substantially identical to the second portion of the one or more second target nucleic acid sequences;~~ and

~~the sample is contacted with two subsets of spectrally-addressable bound probes and one subset of free probes~~ the one or more subsets of spectrally-addressable bound probes comprise a first subset and a second subset, wherein the first subset of spectrally-addressable bound probes is specific for the first portion of the one or more first target nucleic acid sequences, and wherein the second subset of spectrally-addressable bound probes is specific for the first portion of the one or more second target nucleic acid sequences, and wherein the one or more subsets of free probes have substantially identical nucleotide sequences that are specific for the second portion of the one or more first and second target nucleic acid sequences.

4. (Canceled)

5. (Currently amended) A nucleic acid ligation assay, comprising:

contacting a sample suspected of containing one or more target nucleic acid sequences with one or more subsets of free probes and one or more subsets of spectrally-addressable bound probes;

allowing the one or more subsets of free probes and the one or more subsets of spectrally-addressable bound probes to hybridize to the one or more target nucleic acid sequences, if present in the sample;

ligating the hybridized free probes with the hybridized spectrally-addressable bound ~~probe~~ probes, wherein a free probe hybridized to a target nucleic acid sequence is ligated with a spectrally-addressable bound probe hybridized to the same target nucleic acid sequence, to provide spectrally-addressable ligated products; and

~~one or both of detecting the a presence of the spectrally-spectrally-addressable ligated products, or analyzing the target nucleic acid sequence sequences of the spectrally-addressable ligated products, or performing said detecting and said analyzing; and wherein:~~

~~the sample is suspected of containing the one or more target nucleic acid sequences comprise one or more first and one or more second target nucleic acid sequences, wherein the one or more first target nucleic acid sequences have at least a first portion and a second portion, and wherein the one or more second target nucleic acid sequences have at least a first portion and a second portion, and wherein the first~~

portion of the one or more first target nucleic acid sequences is distinguishable from the first portion of the one or more second target nucleic acid sequences, ~~but and wherein~~ the second portion of the one or more first target nucleic acid sequences is substantially identical to the second portion of the one or more second target nucleic acid sequences; and

~~and the sample is contacted with two~~ one or more subsets of free probes comprise a first subset and a second subset, ~~and wherein one subset~~ the one or more subsets of spectrally-addressable bound probes comprise one subset, wherein the first subset of free probes is specific for the first portion of the one or more first target nucleic acid sequences, ~~and wherein the second set of free probes~~ subset is specific for the first portion of the one or more second target nucleic acid sequences, and ~~wherein the one subset of spectrally-addressable bound probes have~~ has substantially identical nucleotide sequences that are specific for the second portion of the one or more first and second target nucleic acid sequences.

6. (Currently amended) An assay according to ~~Claim claim~~ 5, wherein the assay is performed in a first and a second reaction vessel, wherein a first portion of the sample is contacted with the first subset of ~~free probes~~ in the first reaction vessel, and wherein a second portion of the sample is contacted with the second subset of ~~free probes~~ in the second reaction vessel.

7. (Currently amended) An assay according to ~~Claim claim~~ 3, further comprising using a thermostable ligase for ~~said~~ ligating the probes.

8. (Currently amended) An assay according to ~~Claim claim~~ 3, wherein a ~~substantially same amount of at least one fluorescent dye is incorporated into each of the spectrally-addressable~~ bound probes in each subset the first subset comprise a first amount of at least one fluorescent dye, wherein each of the spectrally-addressable bound probes in the second subset comprise a second amount of the at least one fluorescent dye, ~~but wherein each subset of bound probes incorporates a distinctly different amount of fluorescent dye~~ the first amount is different than the second amount, and ~~wherein one the first subset and of spectrally-addressable bound probes is distinguishable from other subsets of spectrally-addressable bound probes~~ the second subset are distinguishable based at least on the relative amount of the at least one fluorescent dye incorporated into the spectrally-addressable bound probe of the subset the first and second amounts.

9. (Currently amended) An assay according to ~~Claim-claim~~ 3, wherein the assay further comprises comprising contacting the sample with polymerase chain reaction components and amplifying the one or more target nucleic acid molecule sequences.

10. (Currently amended) A microsphere-based oligonucleotide ligation assay, comprising:

(a) contacting a sample, which is suspected of containing target nucleic acid molecules ~~having a certain nucleotide sequence~~, with a mixture comprising at least one ~~set-subset~~ of free probes and at least one subset of bound probes, wherein;

(i) ~~the free probes of the at least one subset of free probes of a given set~~ comprise two opposing ends, ~~with wherein a detectable label is at one a first of their the two opposing ends, and wherein a nucleotide is at the a second of the two opposing ends opposite end, and wherein the free probes of the at least one subset of free probes further comprise an oligonucleotide having a predetermined nucleotide sequence that is complementary to at least a first portion of the target nucleic acid molecules;~~

(ii) ~~the bound probes of the at least one subset of bound probes~~ comprise a microsphere and an oligonucleotide probe, wherein the oligonucleotide probes ~~of a given subset of bound probes further comprise an oligonucleotide at one of their ends a first end of the oligonucleotide probes~~ having a modifier moiety, which is used for coupling the oligonucleotide probes to the microspheres, and wherein the oligonucleotide probes further comprises an oligonucleotide having a predetermined nucleotide sequence that is complementary to at least ~~another a second~~ portion of the target nucleic acid molecules; and

(iii) ~~the microspheres of bound probes of a given subset each subset of the at least one subset of bound probes~~ having ~~a an~~ unique spectral address or ~~a an~~ unique fluorescence intensity, which ~~allows one to distinguishes the microspheres of a given subset from those of another different subsets of the at least one subset of bound probes;~~

(b) allowing the at least one subset of free probes and the at least one subset of bound probes to hybridize to the target nucleic acid molecules;

(c) ~~ligating one of the ends of the oligonucleotides of the hybridized free probes with one of the ends of the oligonucleotides of the hybridized bound probes to provide microsphere-bound ligated products; and~~

(d) ~~detecting the presence of the microsphere-bound ligated products.~~

11. (Currently amended) The assay of Claim 10, ~~in which~~ wherein the at least one subset of free probes and the at least one subset of bound probes ~~are allowed to hybridize to different portions of the target nucleic acid molecules.~~

12. (Currently amended) The assay of ~~Claim claim~~ 11, ~~in which~~ wherein the different portions of the target nucleic acid molecules do not overlap.

13. (Currently amended) The assay of ~~Claim claim~~ 10, ~~in which~~ wherein the free probes of the at least one subset of free probes further comprise a phosphate at ~~the other of their~~ the one end of the oligonucleotides of the free probes.

14. (Currently amended) The assay of ~~Claim claim~~ 10, ~~in which~~ wherein the bound probes ~~of the at least one subset of bound probes further comprise a phosphate at the other of their ends~~ the one end of the oligonucleotides of the bound probes.

15. (Currently amended) The assay of ~~Claim claim~~ 10, ~~in which the mixture~~ wherein the at least one subset of bound probes comprises at least two subsets of bound probes, wherein the oligonucleotide probes coupled to the microspheres of ~~one subset~~ the at least two subsets of bound probes being are different from those coupled to the microspheres of the at least one other subset.

16. (Currently amended) The assay of ~~Claim claim~~ 15, ~~in which the bound probes differ in that the nucleotide found at one end of oligonucleotide probes of one subset differs from that found at the corresponding end of the oligonucleotide probes of the other subset~~ wherein a first nucleotide of the predetermined nucleotide sequences of the at least two subsets of bound probes are different, and wherein other nucleotides of the predetermined nucleotide sequences of the at least two subsets of bound probes comprising the oligonucleotide probes of the at least two subsets of bound probes are otherwise substantially identical.

17. (Currently amended) The assay of ~~Claim-claim~~ 16, ~~in which the mixture comprises wherein the free probes of the at least one subset of free probes having have substantially identical predetermined nucleotide sequences.~~

18. (Currently amended) The assay of ~~Claim-claim~~ 15, ~~in which the bound probes differ in the wherein identity-identities of one or more nucleotides at one or more positions of the predetermined nucleotide sequences of the at least two subsets of bound probes are different.~~

19. (Currently amended) The assay of ~~Claim-claim~~ 15, ~~in which the bound probes wherein the at least two subsets of bound probes differ due to one or more substitutions, insertions, deletions, or combinations thereof, at one or more positions of the predetermined nucleotide sequences sequences.~~

20. (Currently amended) The assay of ~~Claim-claim~~ 11, ~~in which the mixture wherein the at least one subset of free probes comprises at least two subsets of free probes, the wherein the nucleotide and the detectable label found at opposite ends of the free probes of one set, first subset of the at least two subsets of free probes differing from the nucleotide and the detectable label found in the corresponding ends of the free probes of the other set, second subset of the at least two subsets of free probes, and wherein the predetermined nucleotide sequences comprising of the at least two sets of free probes first and second subsets are otherwise-substantially identical.~~

21. (Currently amended) The assay of ~~Claim-claim~~ 20, ~~in which the mixture comprises wherein the bound probes of the at least one subset of bound probes having have substantially identical nucleotide sequences.~~

22. (Currently amended) The assay of ~~Claim-claim~~ 10, ~~in which wherein the oligonucleotides of the at least one set-subset of free probes and the at least one subset of bound probes have 5' and 3' ends, and wherein the free probes of a the given set at least one subset of free probes include a phosphate at the 5' ends and a the detectable label at their the 3' ends, and wherein the modifier moiety is an amine which couples the 5' end of the oligonucleotide of the one bound probe to a carboxylic acid group on the microsphere of the same one bound probe.~~

23. (Currently amended) The assay of ~~Claim-claim 22, in which~~ wherein the mixture at least one subset of bound probes comprises at least two subsets of bound probes, and wherein the oligonucleotides coupled to the microspheres of one subset the at least two subsets of bound probes being are different from the oligonucleotides coupled to the microspheres of the other subset of bound probes in that:

a portion of the 3' ends of the oligonucleotides of one subset the at least two subsets of bound probes differs in nucleotide sequence from a portion of the 3' end of the oligonucleotides of the other subset of bound probes; and

wherein other portions the nucleotide sequences comprising of the oligonucleotides of the at least two subsets of bound probes have nucleotide sequences that are otherwise-substantially identical.

24. (Currently amended) The assay of ~~Claim-claim 22, in which the mixture~~ wherein the at least one subset of free probes comprises at least two subsets of free probes, wherein the a portion of the oligonucleotides found at the 5' ends of one set differing from the portion of the oligonucleotide at the 5' ends of the other set the at least two sets of free probes is different, and wherein other portions of the nucleotide sequences of the oligonucleotides comprising of the at least two subsets of free probes are otherwise-substantially identical.

25. (Currently amended) The assay of ~~Claim-claim 22~~, wherein the assay is carried out in a single reaction vessel.

26. (Currently amended) The assay of ~~Claim-claim 22~~, wherein the assay is carried out in separate reaction vessels, using at least one reaction vessel for each ~~set of free probes of the at least one subset of free probes.~~

27. (Currently amended) The assay of ~~Claim-claim 23, in which the microspheres of one subset can be distinguished from the microspheres of the other subset~~ wherein the microspheres of the at least two subsets of bound probes in that the microspheres of the one subset harbor at least one fluorescent dye that emits, upon exposure to an excitatory stimulus, a signal having an intensity that differs from the intensity of a signal emitted by the at least one fluorescent dye harbored by the microspheres of the other subset different intensities.

28. (Currently amended) The assay of ~~Claim-claim 23, in which the spectrally addressable microspheres of one subset can be distinguished from the spectrally addressable microspheres of another subset by the wherein the microspheres of the at least two subsets of bound probes have different~~ relative amounts of at least two fluorescent dyes harbored by the spectrally addressable microspheres.

29. (Currently amended) The assay of ~~Claim-claim 22, wherein the mixture further comprises polymerase chain reaction components, and wherein the assay further comprises the step of amplifying a portion of the target nucleic acid molecule~~ molecules.

30.-34. (Canceled)

35. (Currently amended) The assay of ~~Claim-claim 10, in which~~ wherein the modifier moiety comprises an amine modifier moiety.

36. (Currently amended) The assay of ~~Claim-claim 10, in which~~ wherein the modifier moiety comprises a primary amine group for coupling the bound ~~probe-probes of the at least one subset of bound probes to a~~ carboxylic acid group of the ~~microsphere~~ microspheres.